USEPA Great Lakes Mid-Atlantic Hazardous Substance Research Center

Evalutation of Surfactants for the Enhancement of PCB Dechlorination in Soils and Sediments (SERDP Funded Project)

Final Progress Report

For the Period:
October 1, 1997 to September 30, 2002

OCA Deliverable No. 4
Georgia Tech Project No. E-20-633

February 25, 2003

Principal Investigator: Kurt D. Pennell, Ph.D.
Associate Professor
School of Civil & Environmental Engineering
311 Ferst Drive
Atlanta, GA 30332-0512
Tel: 404-894-9365
Fax: 404-894-8266
e-mail: kpennell@ce.gatech.edu

(I) PROJECT TITLE: Evaluation of Surfactants for the Enhancement of PCB Dechlorination in Soils and Sediments (SERDP Project)

(II) INVESTIGATOR: Kurt D. Pennell, Associate Professor, School of Civil & Environmental Engineering, 311 Ferst Drive, Georgia Institute of Technology, Atlanta, GA 30306-0512.

(III) PROJECT GOALS:

The overall objective of this aspect of the SERDP PCB project (Task B) is to develop and evaluate technologies designed to enhance the bioremediation of PCB-contaminated soils. To achieve this a goal, a matrix of laboratory studies will be conducted to evaluate specific processes governing PCB bioremediation, and to provide the technical basis for subsequent pilot-scale tests of enhanced PCB bioremediation. A primary focus of Task B has been the selection and testing of surfactants to increase the bioavailability of sorbed-phase PCBs, identified as one of the most important factors limiting PCB bioremediation in the field. This approach is based on the ability of surfactants, at concentrations above the critical micelle concentration (CMC), to increase the aqueous solubility of hydrophobic organic compounds including PCBs. Specifically, this research is designed to investigate the processes governing surfactant enhanced bioremediation, ensure compatibility between surfactants and engineered microorganism developed in Task A of the SERDP PCB project (Tiedje), and to conduct laboratory-scale treatability that will serve as the basis for pilot-scale testing of PCB bioremediation in the field.

As noted above, the project is being performed as part of the PCB Thrust Area, in collaboration with Jim Tiedje at Michigan State University and Lance Hansen at the US Army Engineering Research and Development Center (ERDC), formerly known as the Waterway Experiment Station (WES). Dr. Tiedje and Dr. Tsoi supplied the native and engineered bacterial strains and assisted in the development of inoculation procedures for the laboratory tests. Lance Hansen is coordinating field implementation of the bioreactor for treatment of PCB-contaminated soil obtained from a General Electric facility located in Rome, GA.

Research Objectives

1) Investigate the ability of two aerobic bacteria, *Rhodococcus erythreus* sp. NY05 and *Comamonas testosteroni* sp. VP44 , to grow on biphenyl and specific PCB congeners in the presence and absence of three representative nonionic surfactants, Witconol SN-120, Tergitol NP-15 and Tween 80.
2) Quantify the effects of surfactant additions on the microbial transformation of PCB congeners in liquid and solid-liquid systems inoculated with native and engineered strains of *Rhodococcus erythreus* sp. NY05 and *Comamonas testosteroni* sp. VP44 .
3) Investigate the effects of design parameters and operating conditions on rates of PCB transformation in bioreactors. Variables to be considered include surfactant concentration, soil-solution ratio (moisture content), and mixing frequency and duration (continuous vs. intermittent mixing).
4) Develop a mathematical model to describe the coupled sorption/desorption, micellar solubilization, and transformation of PCB congeners under aerobic conditions. The model will incorporate rate-limited sorption processes, rate-limited solubilization, and rates of biotransformation and microbial growth.
5) Design and test pilot-scale biotreatment systems, including bioreactors and landfarming, incorporating specific technologies designed to overcome factors limiting PCB

bioremediation. Perform economic analyses of enhanced PCB bioremediation systems relative to conventional PCB treatment technologies, and address regulatory issues related to the use of genetically engineered microorganisms (GEMs) for PCB bioremediation.

(IV) RATIONALE:

The remediation of PCB-contaminated soils and sediments typically involves excavation of the contaminated material followed by landfill disposal or incineration. The high costs, long-term liability and regulatory issues associated with this approach have reduced the attractiveness of excavation as an ultimate remediation option. In addition, excavation and off-site transport of PCB-contaminated wastes may actually increase the potential for human exposure. Recognition of the potential economic and health implications associated with traditional PCB treatment methods has led to a renewed interest in the development of in-situ and on-site treatment technologies, including enhanced bioremediation processes.

Although microbial transformation of PCBs has been the subject of intense study over the past 25 years, it is now apparent that the use of simplistic remediation approaches, based on conventional bioreactor and landfarming strategies, will not be successful. One of the primary barriers to effective PCB bioremediation is the limited availability of PCBs to microbial populations. PCBs are extremely hydrophobic compounds, which results in their low equilibrium solubilities and slow rates of desorption from solid phases. These physical/chemical barriers may contribute to incomplete bioremediation of PCB-contaminated sites and the inability to reach target PCB concentrations.

To overcome such limitations, we have proposed the use of surfactants to increase the equilibrium solubility and mass transfer rate of PCBs into the aqueous phase. Three commercially-available surfactants were selected for study; Tween 80, Witconol SN-120, and Tergitol NP-15. These surfactants cost approximately $1.00/lb, and thus, surfactant costs for a 5,000 mg/L solution would be approximately $3.78 per ton of soil, assuming a water-filled porosity of 0.3. The research is funded as part of the Strategic Environmental Research and Development Program (SERDP) Federal Integrated Biotreatment Research Consortium (FIBRC). The work is being performed in collaboration with Dr. James Tiedje of Michigan State University and Dr. Walter J. Weber, Jr. of the University of Michigan.

(VI) RESULTS:

The following sections summarize experimental results obtained during the period October 1, 1997 through September 30, 2002. The work performed under Task 1 involved a large matrix of batch experiments that were designed to select

Task 1. Microbial Growth Studies

A matrix of microbial growth studies was conducted to assess surfactant compatibility with two bacterial strains; *Comamonas testosteroni* (VP44) gram (-) and *Rhodococcus erythreus* (NY05) gram (+), supplied by Dr. Tiedje and coworkers at Michigan State University. The gram (+) strain (NY05) has been shown to tolerate high concentrations of PCBs and is likely to withstand anaerobic-aerobic cycling in a sequential reactor system. Three nonionic surfactants were selected to represent a range in solubilization capacity and susceptibility to biodegradation. The surfactants included a linear alcohol ethoxylate (Witconol SN-120), a nonylphenol ethoxylate (Tergitol NP-15) and an ethoxylated sorbitan monooleate (Tween 80). Linear alcohol ethoxylates have been shown to be readily degraded, while nonylphenol ethoxylates are

considered to be recalcitrant. Tween 80 represents an intermediate between these two extremes. A summary of the experimental matrix used for the growth studies is given in Table 1.

Table 1. Matrix of bacteria, substrate and surfactants used in the growth experiments.

Component	**Description**
Microorganisms	*Rhodococcus erythreus* (NY05), *Comamonas testosteroni* (VP44)
Growth Substrate	Biphenyl , 4-chlorobiphenyl (4-CBP), 2,2'-dichlorobiphenyl (2,2'-CBP)
Surfactants	Witconol SN-120, Tergitol NP-15, Tween 80

The bacteria were initially grown in small vials containing 10 mL of K1 media plus biphenyl at concentrations of 30 mM for NY05 and 3 mM for VP44. After reaching the stationary growth phase, ~150 hours at 30°C and 200 rpm, the bacteria seed was transferred to larger growth flasks containing 150 mL of liquid K1 media and biphenyl, surfactant, biphenyl+surfactant or 4-chlorobiphenyl+surfactant. To remove residual biphenyl, the bacteria were rinsed and centrifuged twice prior to transfer. Growth was monitored over time by absorbance at 600 nm using a Varian Cary 3E Spectrophotometer.

Both NY05 and VP44 exhibited rapid growth on biphenyl alone, reaching an optical density of 2-3 after 24 hours. The NY05 strain was grown at equivalent biphenyl concentrations of 30-40 mM, while VP44 was grown at concentrations of 3-6 mM. These findings are consistent with the observed tolerance of NY05 to extremely high concentrations (up to 1000 ppm) of Aroclor 1242. However, neither NY05 or VP44 grew in solutions containing Witconol SN-120, Witconol SN-120+biphenyl and Witconol SN-120+4-chlorobiphenyl. These data indicate that Witconol SN-120 cannot be used as a substrate by NY05 and VP44, and that Witconol SN-120 inhibits utilization of biphenyl and 4-chlorobiphenyl. These results suggest that Witconol SN-120 is unsuitable for use in enhanced PCB bioremediation systems.

Growth of NY05 and VP44 on biphenyl alone, Tween 80, Tween 80+biphenyl, and Tween 80+4-chlorobiphenyl were virtually the same. A slight dependence on surfactant concentration was observed when the concentration of Tween 80 was increased from 125 ppm to 5,575 ppm. Growth of NY05 on biphenyl was not influenced by the presence of Tween 80 at concentrations ranging from 1,200 to 2,500 ppm of surfactant. Similar trends were observed for VP44. These data indicate that Tween 80 can be readily utilized as a food source and does not inhibit growth on biphenyl. However, Tween 80 could be utilized as preferential substrate, and thus, may not be suitable for PCB bioremediation.

For both NY05 and VP44, no growth was observed on Tergitol NP-15 alone over concentrations ranging from 187 ppm to 4,000 ppm. This behavior was anticipated given the reported difficulties in degrading ethoxylated alkylphenol surfactants. However, the observed growth of NY05 and VP44 on biphenyl and 4-chlorobiphenyl in the presence of Tergitol NP-15 at concentrations up to 4,300 ppm, was not anticipated. These finding suggest that Tergitol NP-15 will not be used as a preferential growth substrate by PCB-degrading bacteria and that the presence of Tergitol NP-15 will not inhibit growth on PCB congeners. For these reasons, Tergitol NP-15 may be an ideal candidate for use in PCB bioremediation systems. Examples of microbial growth curves are given in Figure 1, with a summary of the results provided in Table

2.

The most recent series of growth experiments, conducted from April to May 1998, included growth of strain VP44 on both 4-CBP and the di-chlorobiphenyl, 2,2'-CBP. Although these last experiments did not include surfactants, they illustrated the ability of the microorganisms to grow on the para-chlorobiphenyl, 4-CBP, as the sole food substrate. In contrast, no microbial growth was detected in the presence of 2,2'-CBP. These experiments emphasized the need for engineered microbial strains capable of metabolizing chlorobiphenyl compounds with chlorines located on both rings and, in particular, located in the ortho- positions.

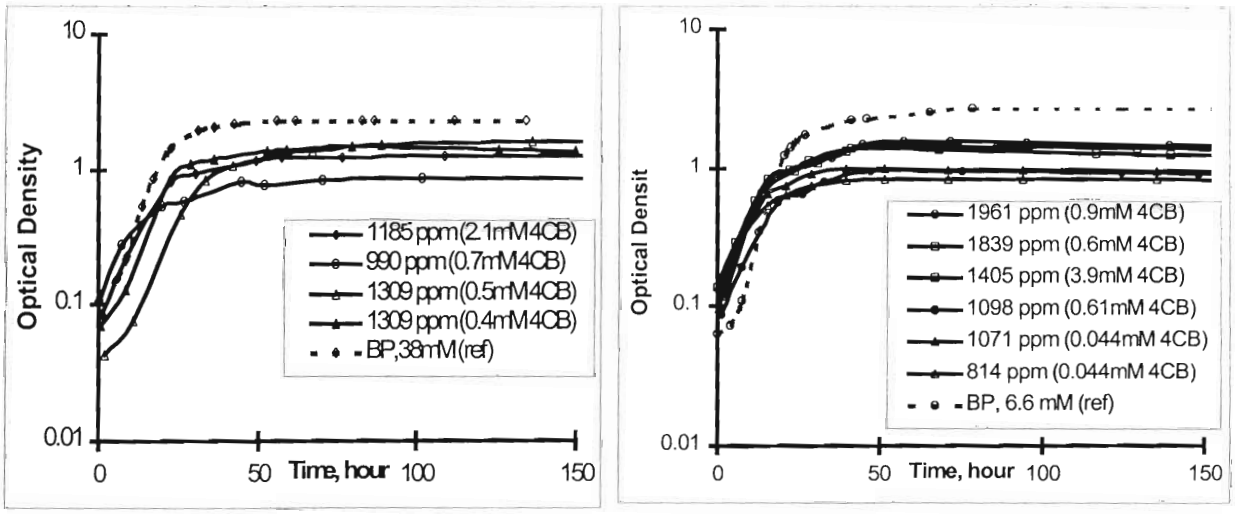

Figure 1. Examples of NY05 and VP44 growth on Tween 80+4-CBP.

Table 2. Summary of results from microbial growth studies.

Substrate	NY05	VP44
Biphenyl	Growth	Growth
4-CBP	Growth	Growth
Witconol SN-120	No Growth	No Growth
SN-120 + Biphenyl	No Growth	No Growth
SN-120 + 4-CBP	No Growth	No Growth
Tween 80	Growth	Growth
T80 + Biphenyl	Growth	Growth
T80 + 4-CBP	Growth	Growth
Tergitol NP-15	No Growth	No Growth
NP-15 + Biphenyl	Growth	Growth
NP-15 + 4-CBP	Growth	Growth

Task 2. Plasmid Stability Studies

A series of experiment was performed to ascertain whether microorganisms engineered to grow on a particular PCB congener or pathway would lose that ability if allowed to grow on other substrates, including surfactants. Initially, solutions of 4-chlorobenzoic acid (4-CBA) and 2-chlorobenzoic acid (2-CBA) were prepared by dissolving each acid in 1 N KOH. The pH of the solution was brought to below 7.0 by addition of 2 N H_2SO_4. A growth flask was then prepared with approximately 100 mL of K1 nutrient solution, and 2-CBA or 4-CBA was added to reach a concentration of approximately 2 mM. The flasks were inoculated with engineered strains of RHA1, NY05, and VP44 provided by MSU, to which 2 mL of 100 mM CBA solution were added each day. Once the solution in the flask reached an optical density of 1.0 or greater, 10 mL of the solution were used to inoculate a second flask containing the same K1 and CBA solution and the process was repeated.

After the second flask reached an optical density of 1.0, the solution was centrifuged and the supernatent was discarded. The biomass was resuspended in K1 and centrifuged again. This process was repeated two more times in an attempt to remove all of the CBA from solution. After the final centrifugation, the biomass was resuspended to a concentration of 0.2 g biomass per ml. This solution was equally divided and used to inoculate three growth flasks, the first containing a solution of K1 and biphenyl, the second containing a solution of Tergitol NP-15 and biphenyl, and the third containing a solution of Tween 80. A sequential plating method (five plates) was employed, with the fifth plate containing individual colonies. These plates were labeled, sealed with parafilm and forwarded to MSU for analysis. A summary of the PCR analysis for RHA1+fcb is given in Table 3. Notice that under all of the growth conditions tested, the plasmid gene was detected by PCR analysis.

Table 3. Representative plasmid stability test results.

Substrate	Plasmid gene	Initial Substrate	PCR Analysis
Biphenyl + K1	RHA1+fcb	4-CBA	5/5
Tween 80 + K1	RHA1+fcb	4-CBA	5/5
TNP15 + K1	RHA1+fcb	4-CBA	5/5

Task 3. PCB Surfactant Solubilization Experiments

Surfactants are commonly known to enhance the apparent solubility of hydrophobic organic compounds, such as chlorinated biphenyls, at concentrations above the surfactant critical micelle concentration (CMC). The monochlorinated biphenyl, 4-CB, has an aqueous solubility of 2 ppm (0.011 mM). The solubilization capacities of two surfactants, Tween 80 and Tergitol NP-15 were determined foi 4-CB from batch solubility experiments conducted in 26-mL glass serum tubes. The lower portions of the tubes were initially coated with 4-CB, using a solution consisting of 4-CB+hexane. Surfactant concentrations were prepared within the range of 50 to >2000 ppm and were added to the serum tubes, which were placed on an orbital shaker at 150 rpm for two to three weeks. The solubility experiments were conducted at 22° C (room temperature) and 30° C (temperature of microbial growth studies).

After two to three weeks, 0.4-mL samples were taken from the serum tubes and mixed with 1.2 mL of isopropanol in chromatography vials, which were analyzed using gas chromatography with an electron capture detector (GC/ECD). Alternatively, for lower concentrations of 4-CB, samples from the serum tubes were transferred as 10 to 15 mL aliquots to new serum tubes, to

which hexane were added in small volumes of 3 to 5 mL. The serum tubes were centrifuged at 3000 rpm to extract 4-CB from the aqueous solution into the hexane, which was transferred to chromatography vials for GC/ECD analysis.

The solubilization capacity of a surfactant, for a given compound, can be described as molar *solubilization ratio (MSR), which expresses the amount of 4-CB solubilized per amount of* surfactant, on molar basis. Results from solubility experiments for both surfactants are presented in Figure 2.

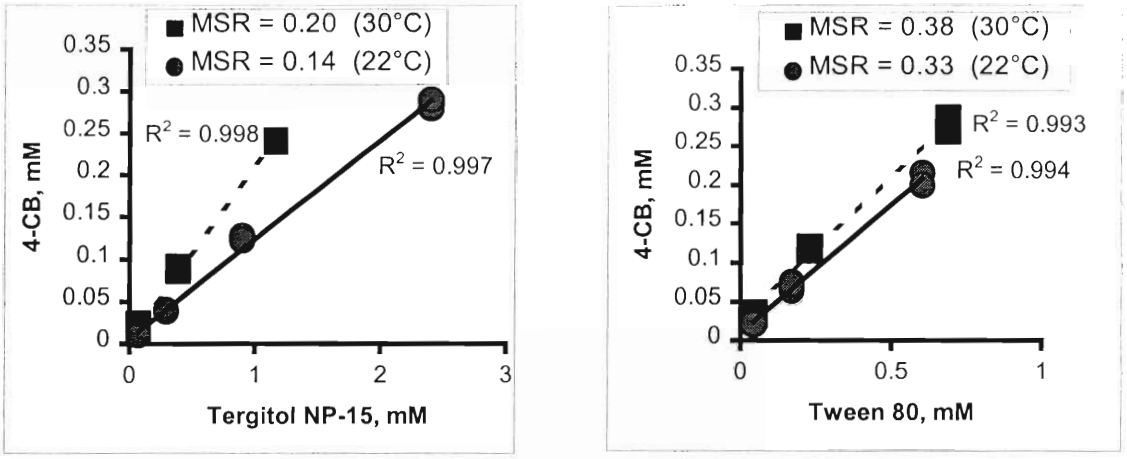

Figure 2. Solubility enhancements of 4-CB in solutions of Tween 80 and Tergitol NP-15 at 22°C and 30°C.

Task 4. PCB Transformation Experiments

A series of PCB and surfactant degradation experiments was conducted to evaluate the ability of the organisms to transform PCB congeners in the presence and absence of surfactant. The microorganisms were first grown in two batches of liquid solution containing biphenyl in order to reactivate microbial growth. The first batch, or small growth, was conducted in 10 mL liquid solution plus biphenyl and was allowed to continue for three to five days. The entire contents of these vials were transferred to the second batch of growth flasks, or large growth, which consisted of 150 mL liquid solution plus biphenyl. This growth cycle was continued for up to five days after which the entire contents of the large growth flasks were centrifuged and rinsed repeatedly to remove residual traces of biphenyl. The resulting microbial seed was resuspended to 0.2 g/mL before being distributed evenly among the prepared degradation flasks. This last set of flasks contained surfactant solution both with and without 4-chlorobiphenyl. As a reference, flasks were included which contained biphenyl alone and 4-chlorobiphenyl alone. Growth in all of the flasks was monitored by recording the optical density at 600 nm using a UV-Vis spectrophotometer. In addition, samples were collected to measure surfactant and PCB disappearance. Sample aliquots were transferred from the degradation flasks to centrifuge tubes, sulfuric acid was added to the tubes to inhibit further microbial growth, and the tubes were centrifuged at 3000 rpm for 30 minutes. Supernatant from these tubes was transferred to 1.8 mL chromatographic vials which were stored in the refrigerator until analysis on Hewlett Packard 1100 series HPLC. Samples containing Tergitol NP-15 were run using a C-18 column (Hewlett Packard ODS Hypersil, 5 μm, 125 x 4 mm) with the surfactant detected by diode array detector (DAD) at 224 nm and the 4-chlorobiphenyl detected at 262 nm. Those samples containing

Tween 80 were run on a C4 column (Alltech Kromasil, 5 μm, 100 x 4.6 mm) with surfactant detection through an evaporative light scattering detector (ELSD) and 4-CB detected on DAD at 262 nm. A summary of the degradation experiments and their respective components is given in Table 4. The results from a degradation experiment with Tergitol NP-15 and 4-CB are presented in Figure 3. Typically, the 4-CBP concentrations disappeared within two to three days after the addition of microorganisms to the flasks. Concurrent with this disappearance is the appearance of 4-chlorobenzoic acid (4-CBA) which is the dead-end product of 4-CBP metabolism.

Table 4. Summary of PCB degradation experiments conducted with NY05 .

Name Date	**Surfactant Type / Conc.**	**Surf. with 4-chlorobiphenyl**			**4-CBP Alone**	**Biphenyl Alone**
		Low	**Mid**	**High**		
DegN1 5/23/98	Tergitol NP-15 1730 ppm	1.1 mM	4.3 mM	5.6 mM	4.7 mM	69.6 mM
DegN2 6/23/98	Tergitol NP-15 1750 ppm	0.6 mM	4.6 mM	5.6 mM	4.6 mM	63 mM
DegN3 7/11/98	Tween 80 4680 ppm	1.4 mM	5.2 mM	5.8 mM	5.3 mM	56.9 mM
DegN4 8/2/98	Tergitol NP-15 1500 ppm	2.6 mM			3.1 mM	
	Tween 80 1380 ppm	2.9 mM				
DegN5 8/18/98	Tergitol NP-15 1750 ppm	2.1 mM			2.1 mM	
	Tween 80 1640 ppm	2.1 mM				

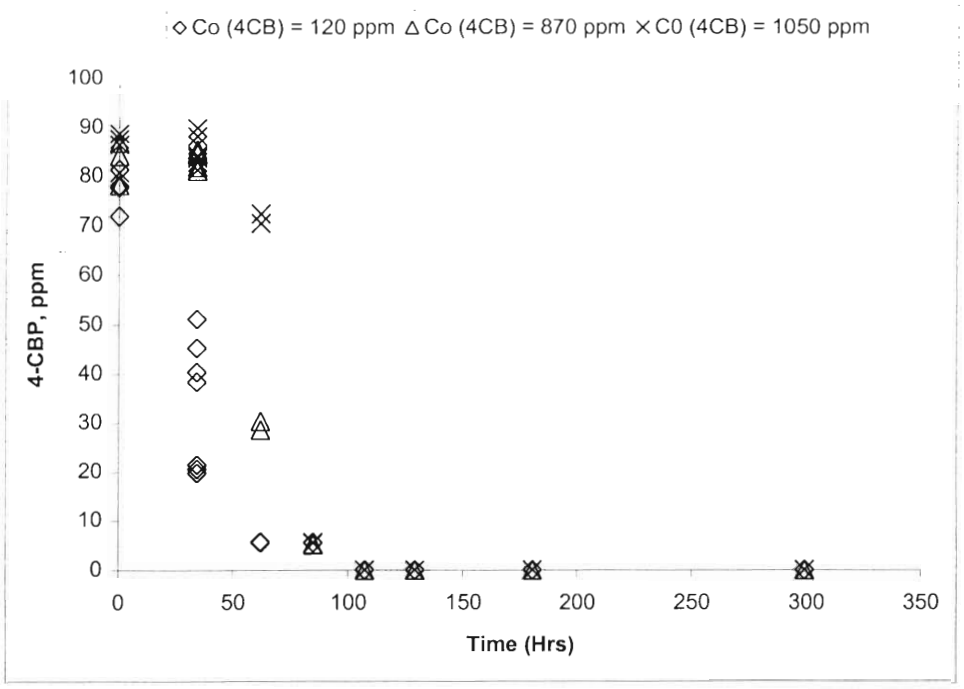

Figure 3. Transformation of 4-CBP by NY05 with 1750 mg/L Tergitol NP-15.

The microbial growth experiments confirmed the ability of biphenyl-degrading microorganisms to achieve substantial growth in solutions of surfactant that contained monochlorinated biphenyl (4-CB). The disappearance of 4-CB from liquid batch cultures was monitored through additional experiments that were designed to address the fate of both the surfactants and the chlorinated biphenyls. These transformation experiments were conducted in 300-mL glass culture flasks that were sterilized and coated with 4-CB. An aqueous solution of nutrient K1 or solutions of surfactant, either Tergitol NP-15 or Tween 80, was added to the prepared flasks and allowed to mix for ten to thirteen hours at 30° C, 150 rpm. Resting cells of strain NY05, which had been rinsed three times to remove residual biphenyl, were suspended in K1 at 0.2 g/mL and distributed among the flasks to begin the transformation experiments. Samples were transferred from the flasks to 26-mL glass serum tubes to which was added sulfuric acid (2N H_2SO_4) in order to lower the pH to about 3.5 and halt microbial activity. Additional preparation of the samples included hexane extractions for flasks that did not contain surfactant solutions, and centrifugation to remove biomass. Samples were then transferred to chromatography vials for analysis by GC/ECD. Results from representative degradation experiments are presented in Figure 2.4 for surfactant and K1 solutions.

In order to confirm that the disappearance of 4-CB was microbially-mediated, controls were included in the transformation experiments. The control flasks, both with and without surfactant solutions, were not inoculated with NY05 resting cells. These controls enabled detection of possible abiotic influences, such as volatilization or adsorption to the flasks, which would have been interpreted as 4-CB degradation. Representative results obtained for abiotic controls, for both surfactants, are presented in Figure 4.

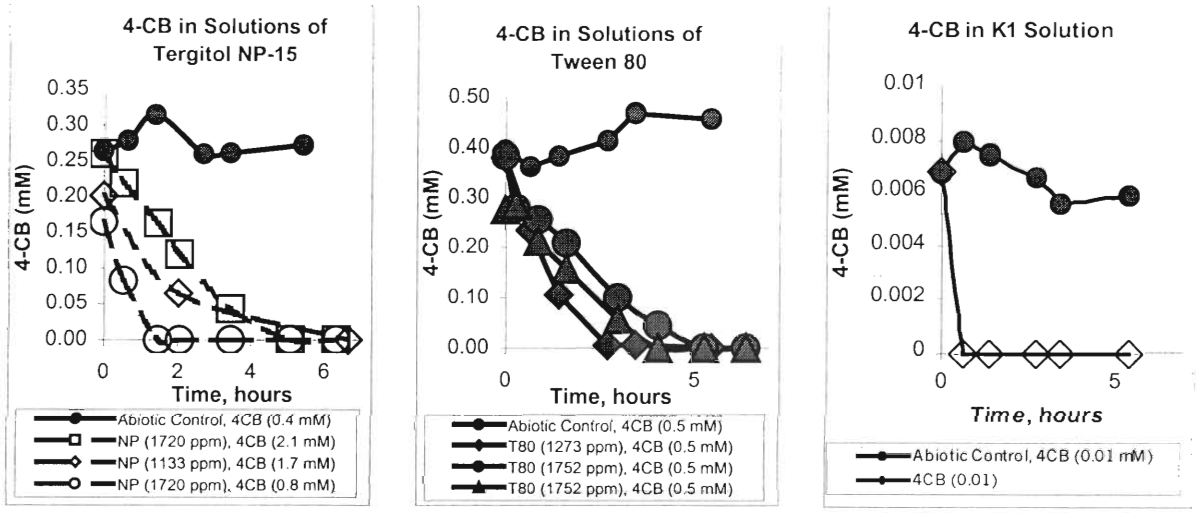

Figure 4. Transformation of 4-CB by *Rhodococcus* NY05 in solutions of Tergitol NP-15, Tween 80, and K1 nutrient medium.

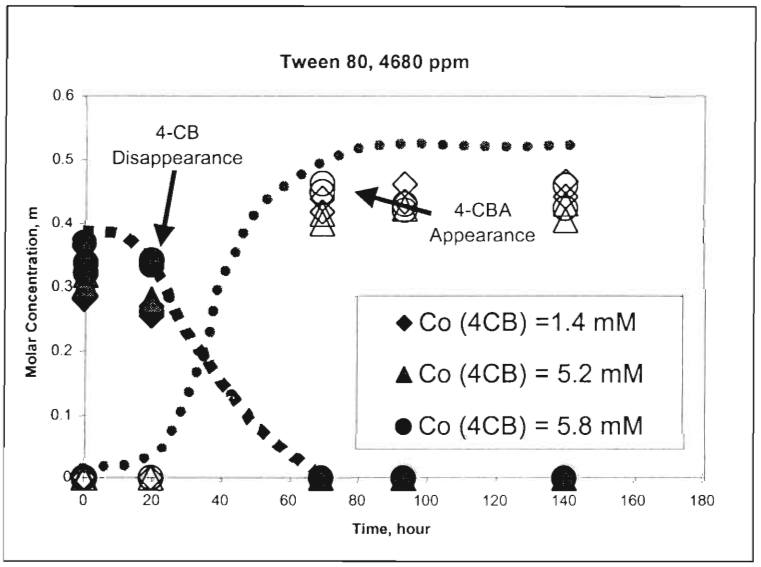

Figure 5. Disappearance of 4-CB and production of 4-CBA by *Rhodococcus* NY05 in a Tween 80 solution (4,680 mg/L).

In addition, the transformation of 4-CB by strain NY05 has been shown to result in the production of stoichiometric amounts of the corresponding chlorinated benzoic acid, 4-CBA. The detection of this metabolite provided additional confirmation that the disappearance of 4-CB was microbially mediated. The production of 4-CBA, for a representative degradation experiment conducted in a solution of Tween 80, is shown in Figure 5. The degradation phase of the experiment was initiated well before micellar solubilization approached equilibrium, and as such, the molar concentration of 4-CBA was greater than the initial amount of 4-CB in solution.

Task 5. Batch Reactor Sorption Experiments

A series of solid-liquid phase experiments was conducted using small (25mL) batch reactors and three larger-scale (500 mL) intermittent mixing reactors. The initial surfactant sorption studies were performed using 25 ml glass or Nalgene vials with teflon seals. Surfactant solutions were prepared at six different concentrations with a background solution of 500 ppm $CaCl_2$ as well as 500 ppm sodium azide to prevent bacterial growth. Five reactors were used for each surfactant concentration, with three containing soil or sand and the remaining two serving as blanks. Approximately 20 ml of solution, with 1 to 10 grams of soil or sand, were placed in each vial. The reactors were then placed on shaker tables for a sufficient amount of time to allow the surfactant to reach an equilibrium concentration between the sorbed and aqueous phases (in most cases 2 weeks). The reactors were then removed from the shaker tables and those with soil were centrifuged. The supernatent analysis was performed using an HPLC method to determine aqueous concentrations of the surfactant. A summary of the batch sorption experiments is given in Table 5.

Desorption studies were undertaken using the Appling soil to determine if there was a difference in the sorption and desorption isotherms. The experiments were performed in the same way as the other batch experiments; however, once the reactors had been centrifuged, approximately 10 ml of supernatent were removed and replaced with background solution. The soil was resuspended and the reactors were then returned to the shaker tables until they reached a new equilibrium concentration.

Table 5. Summary of surfactant sorption experiments.

Soil	% Org. Carbon	Amt of Soil Used	Surfactants Used	No. of trials	Min. Shaking Time
Ottawa sand (20-30 mesh)	NA	10 grams	Tergitol NP-15	1	4 days
Appling soil	0.75	1 gram	Tergitol NP-15 Tween 80	2 2	14 days
WES soil	0.35	5 grams	Tergitol NP-15 Tween 80	2 1	8 days

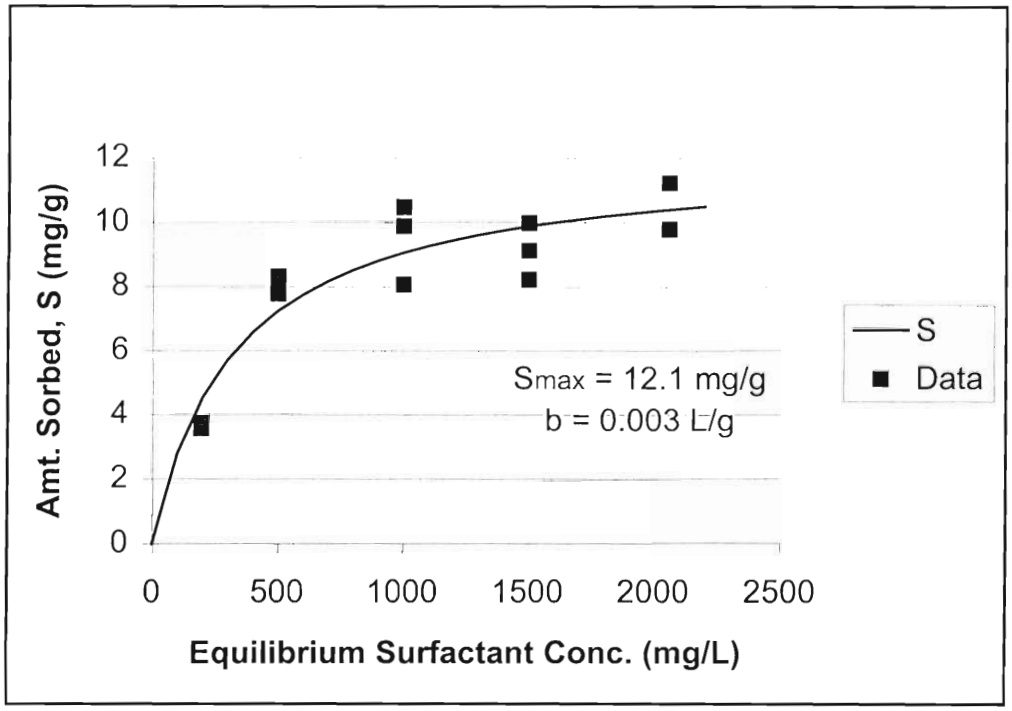

Figure 4. Sorption of Tergitol NP-15 by WES soil.

Task 6. Mathematical Modeling

In order to predict the effect of surfactant additions on the distribution of PCB congeners in a solid-liquid system, it is necessary to account for the potential impact of sorbed-phase and micellar surfactant on PCB bioavailability. Surfactant micelles will act to increase the amount of PCB in solution, however, sorbed-phase surfactant may also increase partitioning of the PCB to the solid phase. This effect will be a function of the surfactant critical micelle concentration (CMC), the soil sorptive capacity (Sm), and the partitioning of the PCB congener among the aqueous, micellar and solid phases. Conceptually, the overall or apparent solubility of a compound in the presence of surfactant can be represented as the amount of solute associated with surfactant monomers plus the amount associated with surfactant micelles. This can be expressed in the following form:

$$C_{PCE}^{*}/C_{PCE} = 1 + C_{monomer}K_{monomer} + C_{micelle}K_{micelle}$$

where, C_{PCE}^{*} is the apparent solubility of solute at the total surfactant concentration (mg/L), C_{PCE} is the intrinsic solubility of solute in pure water (mg/L), $C_{monomer}$ is the concentration of surfactant monomers (mg/L), $C_{micelle}$ is the concentration of micelles (mg/L), $K_{monomer}$ is the solute distribution coefficient between surfactant monomers and water (L/mg), and $K_{micelle}$ is the solute distribution coefficient between the micelles and water (L/mg). This approach can be extended to include the effect of sorbed-phase surfactant on the distribution of solute between the solid and aqueous phases.

$$K_{PCE}^{*} = K_{PCE}(1 + C_{s/om}K_{s/om})/(1 + C_{monomer}K_{monomer} + C_{micelle}K_{micelle})$$

where, K^* is the apparent soil-water distribution coefficient (L/kg), K is the intrinsic soil-water distribution coefficient (L/kg), $C_{s/om}$ is the concentration of sorbed surfactant per unit mass of native soil organic matter, $K_{s/om}$ is the solute distribution coefficient between sorbed surfactant and organic matter (K_s/K_{om}), and Ks is the solute distribution coefficient between the sorbed surfactant and water.

The above equation was incorporated into a macro-based spreadsheet to investigate the effects of system parameters on the overall or apparent distribution coefficient (K*) of DDT as a function of surfactant concentration. Using the measured data reported above, the sensitivity of K* to $K_{s/om}$ was investigated over a surfactant concentration range of 0 to 800 mg/L (Figure 5). The model will be adapted to account for rate-limited sorption and desorption of both the surfactant and the PCB congener. In addition, experimental data were collected to evaluate the ability of the model to predict the coupled sorption of PCBs and surfactant.

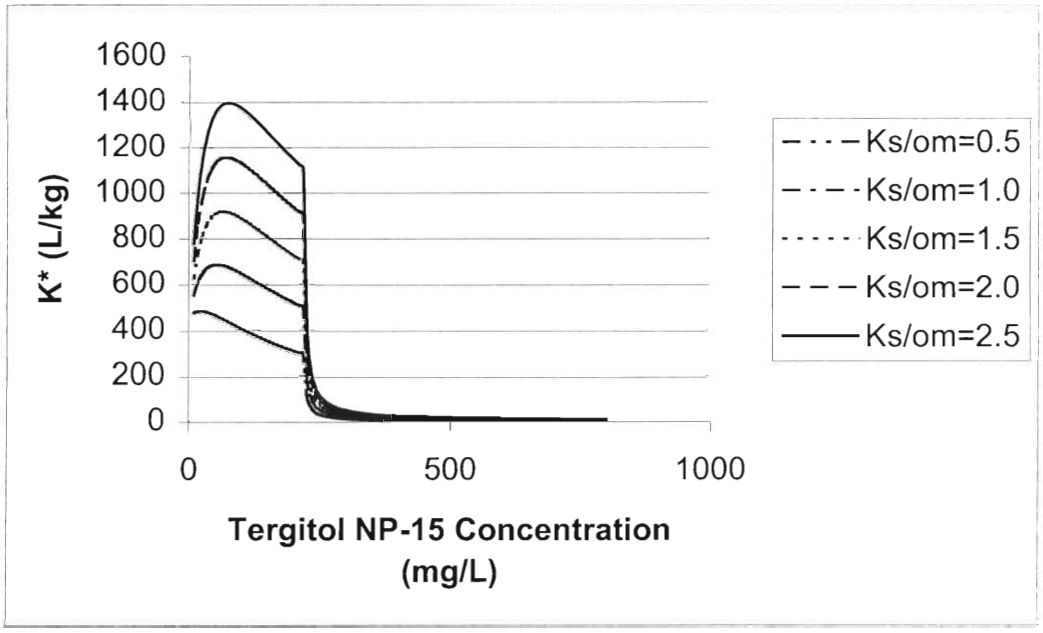

Figure 5. Effect of variations in the value of $K_{s/om}$ on the overall distribution coefficient.

Task 7. Cost Analysis of PCB Remediation Technologies

A detailed literature review was performed to develop a database for PCB remediation technologies. This information was summarized in 70-page report which presents the regulations governing PCB treatment and disposal, describes available treatment options, and compares reported costs for each treatment alternative. As anticipated, it was found that incineration remains a widely used treatment technology despite relatively high costs, ranging from $1.65 to $6.60 per kg of contaminated soil. Other technologies gaining increased attention include thermal desorption, chemical dehalogenation, solidification-stabilization, chemical extraction, landfarming and biopiling. A summary of the advantages, disadvantages and cost-range is given in Table 6.

Task 8. Regulation of Genetically Engineered Microorganisms (GEMs)

A second report was generated which summarized regulations governing the use of genetically engineered microorganisms (GEMS). The final rules for EPA supervision of GEMs under Section 5 of the Toxic Substances Control Act (TSCA) were published in 1997 (part 725, Title 40, Code of Federal Regulations). Research and development of GEMs that takes place within contained structures, whether commercial or otherwise, are exempt from filing a Microbial Commercial Activity Notice (MCAN) if the research is conducted under mandatory compliance with National Institute of Health (NIH) guidelines for "Research Involving Recombinant DNA Molecules" or if records are maintained that demonstrate voluntary compliance. The NIH guidelines specify the practices to be followed for physical containment, ranging from Biosafety Level 1 (BL1) to Biosafety Level 4 (BL4). Each containment protocol includes a set a standard practices as well as a description of special procedures, equipment and installations. In addition to adherence to the NIH Guidelines, activities that qualify for the contained research exemption must be conducted under the supervision of a Technically-Qualified Individual (TQI). The TQI is required to specify and substantiate appropriate procedures for controlling access to the facility, inactivating microbes in the waste stream and controlling fugitive emissions of microorganisms.

For non-contained research activities, a TCSA Experimental Release Application (TERA) can be filed with the EPA in lieu of an MCAN. Although TERAs require less information than MCANs, all available test data regarding health and environmental effects must be submitted. The EPA has 60 days, extendible to 120 days, to review the TERA, and may specify restrictive conditions. A flow chart outlining the regulatory approval steps is given in Figure 6. Unless a field-scale application is undertaken, the research conducted in this project will fall under the contained structures exemption. Dr. Tiedje will serve as the TQI for such activities.

Table 6. Comparison of PCB Treatment Technologies

Technology	Advantages	Disadvantages	Estimated cost per kg
Incineration	• Destroys PCBs • 99.9999% DRE • Quick; well-documented	• Expensive • Possible dioxin formation • Public opposition	$1.65 - $6.60
Thermal desorption	• Insensitive to co-contaminants • Effective at high PCB concentrations	• PCBs not destroyed • Soil sterilized • High clay and moisture content decrease efficiency	• *In situ:* $0.02 - $0.08 • *Ex situ*: $0.045 - $0.33
Chemical dehalogenation	• Destroys PCBs • Stand-alone technology	• High PCB concentrations require more chemicals • Size reduction required • High clay and moisture content decreases efficiency	• BCD:$0.11 • APEG: $0.22 - $0.55
Solidification/ Stabilization	• Can be implemented *in situ* • Effective on inorganic co-contaminants	• PCBs not destroyed • Contaminant leaching possible • Long-term monitoring required	$0.011
Vitrification	• Destroys PCBs • Effective on co-contaminants • Effective on wide range of soils	• Sensitive to soil moisture content • Off-gases must be treated	$0.16 – $0.47
Soil washing	• Effective on co-contaminants • No off-gases	• PCBs not destroyed • Sensitive to ambient temperature	$0.02 - $0.19
Chemical extraction	• Effective at high PCB concentrations • Insensitive to co-contaminants	• PCBs not destroyed • High capital costs • High clay and moisture content decrease efficiency	• Solvent $0.11 -$0.44 • SFE: $0.12 - $0.19

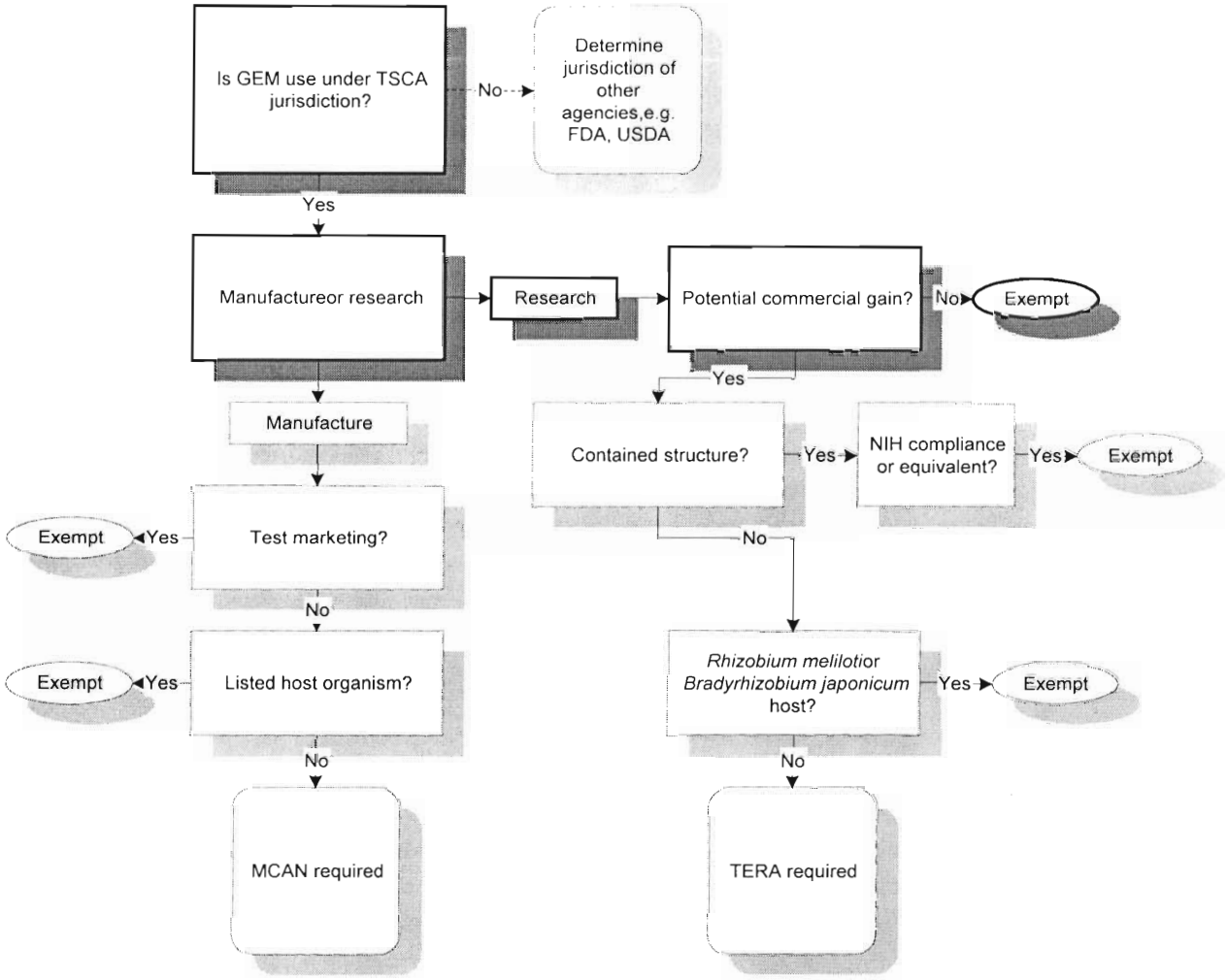

Figure 6. Flow chart illustrating regulatory procedures applicable to GEMs.

Task 9. Bioreactor Design

A pilot-scale test was designed to incorporate side-by-side comparisons of three bioremediation options. The tests were performed at the Environmental Laboratory, Waterways Experiment Station (WES), USACE Engineer Research & Development Center (ERDC), Vicksburg, MS. Three parallel reactors with different solids loading rates will be operated to evaluate the effects of solids loading on soil mixing, aeration, addition and uniform distribution of nutrient amendments/GEMs. These factors will in turn influence the rate of bioremediation. The three solids loading rates that will be investigated are:

- Low solids loading (≈ 5 – 10% wt.): A traditional slurry reactor will be used for low solids loading soil slurry. The slurry reactor is a 400 gallon stainless steel tank with cone shaped bottom for convenient loading. The reactor is fitted with an agitator for mixing. With a free board of approximately 30 %, the reactor can hold 280 gallons (1058 L).

- Medium solids loading (≈ 45 – 60% wt.): A medium solids soil slurry will be treated in a novel shaftless screw reactor. The reactor is fitted with three shaftless screws to mix and convey the high solids slurry. The main benefit of the shaftless screw reactor is reduced water content relative to a traditional slurry reactor, which avoids the dewatering costs of the stabilized soil. Shaftless screw reactor, with dimensions of approximately 3.1 ft. x 1.9 ft. x 1.3 ft. will have a working volume of about 4.4 cft. The slurry in this reactor will be about 9 inches thick to ensure uniform mixing and to avoid dead spaces.

- High solids loading (> 90% wt.): This will represent a contaminant treatment similar to *in situ* landfarming contained in an oval shaped reactor. The reactor is made of galvanized iron, with dimensions of 4 ft. x 2 ft. x 2 ft. Contaminated soil at a moisture content of approximately 8% (w/w) will be treated in this reactor. No water will be added to the soil, except to maintain the moisture content for optimum biological growth (50 – 80% of Field Moisture Capacity [FMC]). The working volume of the high solids reactor will be approximately 6.3 cft.

Nutrient levels (and moisture content in high solids reactor) will be monitored and adjusted weekly for the duration of the study. The material in each reactor will be analyzed at the beginning, end, and at routine intervals for PCB concentration, moisture content, pH, temperature, nutrient levels (N, P, K), oxygen concentration, and microbial biomass. Additionally, initial and final characterization will include particle size distribution (PSD), Atterberg properties, and leachability. The study will evaluate the effects of different solids loading rates and treatments on the bioremediation of PCBs using GEMs as indicated in Table 7.

Each system will undergo a sequential anaerobic-aerobic phase treatment. For the anaerobic phase, all the three reactors will be seeded with the inoculum collected from Hudson River, NY. Anaerobic inoculum will consist of a 1% (w/w dry solid:solid basis) of Hudson river sediment. The inoculum will be applied with 10 mM $FeSO_4$. During the anaerobic phase (1 µL ethanol/L soil treated) will be added to all the three reactors, as a carbon source for microorganisms. 5,000 mg/L of surfactant will be added to the high solids reactor in the beginning of the anaerobic phase while flooding the reactor for removing soil oxygen. After completion of the anaerobic phase, the reactors will be inoculated with GEMs (LB400 and RHA1). Vermiculite impregnated with GEMs will be applied at the rate of 3% (w/w vermiculite:dry solids) of contaminated soil in each reactor. The anaerobic phase transition time will be 6-8 months or a predetermined % dechlorination, which ever comes first. The aerobic phase will follow the anaerobic phase and

will last for about 2-4 months. Since the transition between anaerobic and aerobic phases may be dependent upon PCB congener concentration (degree of chlorination), the precise duration of the demonstration may vary. After each sampling event, the soil samples will be shipped to MSU for contaminant and microbial analysis, and to Georgia Tech for surfactant analysis.

The sample analysis plan is subdivided chronologically throughout the duration of the study as initial sampling, intermediate sampling after every two weeks, and final sampling at the end for each phase (Table 8). To ensure quality control of analytical results, samples will be analyzed in composite replicates of 7 for high solids, 5 for medium solids and 3 for low solids reactors in the beginning and at the end of both phases. Higher replicates are used in high solids reactor because of the level of heterogeneity and variability as compared to well mixed low solids reactor.

Table 7: Experimental Design for Pilot-Scale Bioreactor Tests.

Reactor	Solids Loading	No. of Treatments	Treatments		
Low solids	5 – 10 %	3	Surfactant; Iron + Ethanol	Iron + Ethanol	No amendments
Medium solids	45 – 60 %	1	Iron + Ethanol		
High solids	> 90 %	3	Ethanol	Iron + Ethanol	No amendments

Table 8: General Sample Analysis Plan

Anaerobic Phase		Transition	Aerobic Phase	
Initial Sampling ($t = 0$)	Intermediate Sampling ($t = n_1 \rightarrow t = n_{11}$)	Initial Sampling ($t = 0$)	Intermediate Sampling ($t = n_1 \rightarrow t = n_2$)	Final Sampling ($t = n_3$)
PCB conc.	PCB conc.	PCB conc.	PCB conc.	PCB conc.
Nutrient conc.	Nutrient Conc.	Nutrient conc.	Nutrient conc.	Nutrient conc.
PSD	PH	PH	pH	PSD
PH	Moisture content	Moisture content	Moisture content	pH
Moisture content	Temperature	Microbial biomass	Temperature	Moisture content
Bulk Density		TOC		Bulk Density
Leachability		Metals		Leachability
Microbial biomass		Temperature		Microbial biomass
TOC				TOC
Metals				Atterberg limits
Atterberg limits				Temperature
Temperature				

Task 9. Identification and Collection of PCB-Contaminated Soils

Two sites were initially considered for field activities and collection of contaminated soils, Lake Ontario Ordinance Works (LOOW), near Niagara Falls, NY and the Picatinny Arsenal in north central New Jersey. The first pilot-scale test was planned for the LOOW site during FY00. During excavation of a former TNT waste sewer line at LOOW, PCB contamination was detected. The total volume of contaminated material was estimated to be approximately 27 yd^3. Two 1-gallon buckets of the material were delivered to Georgia Tech and MSU in December 1998. Dr. Tiedje's research group performed analysis of PCB contamination at MSU. It was found that the sample contained approximately 1,000 mg/kg of total PCB, with a congener pattern most similar to a mixture of Aroclor 1242 and Aroclor 1248. Organic carbon analysis of the sample, performed at Georgia Tech, indicated a TOC content of approximately 40% by wt. As delivered, the material appeared as a thick black muck, with a solids loading of approximately 19% on a volume basis and 38% on a weight basis. Unfortunately, subsequent site investigations found no PCB contamination along the pipeline, and thus this effort had to be abandoned.

Subsequent activities are focused on PCB-contaminated soils obtained from the Picatinny Arsenal Site (New Jersey). PCB concentration, primarily as Aroclor 1260, has been found at concentrations ranging from 50 to 75 mg/kg. The soil contains about 3% organic matter, and does not contain any known heavy metals or petroleum hydrocarbons. The total volume of soil needed to run 4 treatment reactors for low solids loading, three treatment reactors for high solids loading and a single treatment reactor for medium solids loading, is about 0.9 – 1.1 cy..

Since the material will be excavated, and possesses a rather low solids loading, it was decided that an ex-situ bioreactor would be used to treat the PCB-contaminated material. The use of an ex-situ bioreactor also allows for greater control over system parameters, and for the containment of genetically engineered microorganisms (GEMs). The latter condition will minimize the number and extent of regulations that must be addressed in order to test the GEMs.

Research activities during the period April 2001 through September 2001 focused on the following initiatives: (1) obtain PCB-contaminated soil from a former General Electric transformer plant (closed in 1998) located in Rome, GA and (2) provide technical and analytical support for the pilot-scale bioreactor experiments performed at WES. Identification and collection of PCB-contaminated soil was initiated by the PI (Kurt Pennell) after failure to obtain suitable materials from at least three DOD facilities, including Lake Ontario Ordnance Works (LOOW), Picatinny Arsenal, and a former munitions facility in the Baltimore District. The key contacts from General Electric are Kevin Holtzclaw (Manager, PCB Issues, GE Corporate, Fairfield, CT) and Robert Witsell (EHS GE Industrial Systems, Rome, GA). The site was originally identified as a candidate by Ms. Jennifer Kaduck (Chief, Hazardous Waste Management Branch, Georgia Environmental Protection Division). Subsequent discussions with GE personnel revealed that the site did indeed contain Arochlor 1242 and 1248 contamination at relatively high levels (> 1,000 mg/kg). The highest concentrations of 1242/1248 were observed in samples collected from a former ditch that runs parrellel to the old railroad tracks, just south and southwest of the Georgia Power substation. Based on this information, the PI collected samples in the vicinity of borehole SB9B-5 in late October, 2000. Analytical results indicated total PCB concentrations ranging from approximately 1 to 950 mg/kg, primarily Arochlor 1242.

In early May 2001, PCB-contaminated soil was collected from the Rome, GA site for use in the pilot-scale bioreactor studies (conducted at WES). The collection effort was coordinated on site by Mr. Roy Wade of WES. Approximately 2 m^3 (500 gallons) of contaminated soil was excavated using a backhow. The material was homogenized and screened on site, and placed in ten 55 gallon drums for shipment to WES.

Task 10. Analytical Support of Pilot-Scale Bioreactors

Research activities undertaken as part of Task 10 focused on providing technical and analytical support for the pilot-scale bioreactor experiments performed at the U.S. Army Engineering Research and Development Center (ERDC). Three parallel bioreactor studies were established to evaluate the effects of solid loading (8, 54 and 73% w/w) and amendments (e.g., nutrient addition) on the bioremediation of a PCB-contaminated soil. A process diagram illustrating the bioreactor treatments is shown in Figure 7. The PCB-contaminated soil was collected from a General Electric facility located in Rome, GA. Approximately 2 m^3 (500 gallons) of contaminated soil was excavated using a backhow, and the material was homogenized and screened on site prior to shipment to ERDC. The initial concentration of PCBs in the soil ranged from 800 to 1000 mg/kg, composed primarily of Aroclor 1242 and 1248.

Samples were collected from each bioreactor on regular intervals and shipped to Georgia Tech for analysis of surfactant (Tween 80) and organic carbon content (TOC). Analysis of PCB concentrations was performed on duplicate (matched) samples by Dr. John Quensen at Michigan State University. Results of the Tween 80 and TOC analyses for samples collected after 0, 76, 136 and 197 days of operation are shown in Tables 9, 10, 11, 12, 13 and 14, respectively. The TOC values for all reactor samples ranged from 5 to 10 mg/g, and remained relatively constant over the sampling periods. Surfactant could only be analyzed for samples containing an aqueous (liquid) phase (i.e., LSR), with values decreasing from about 125 mg/L to 30 mg/L. The observed descrease was most likely due to biodegradation of Tween 80, which has been reported for other systems.

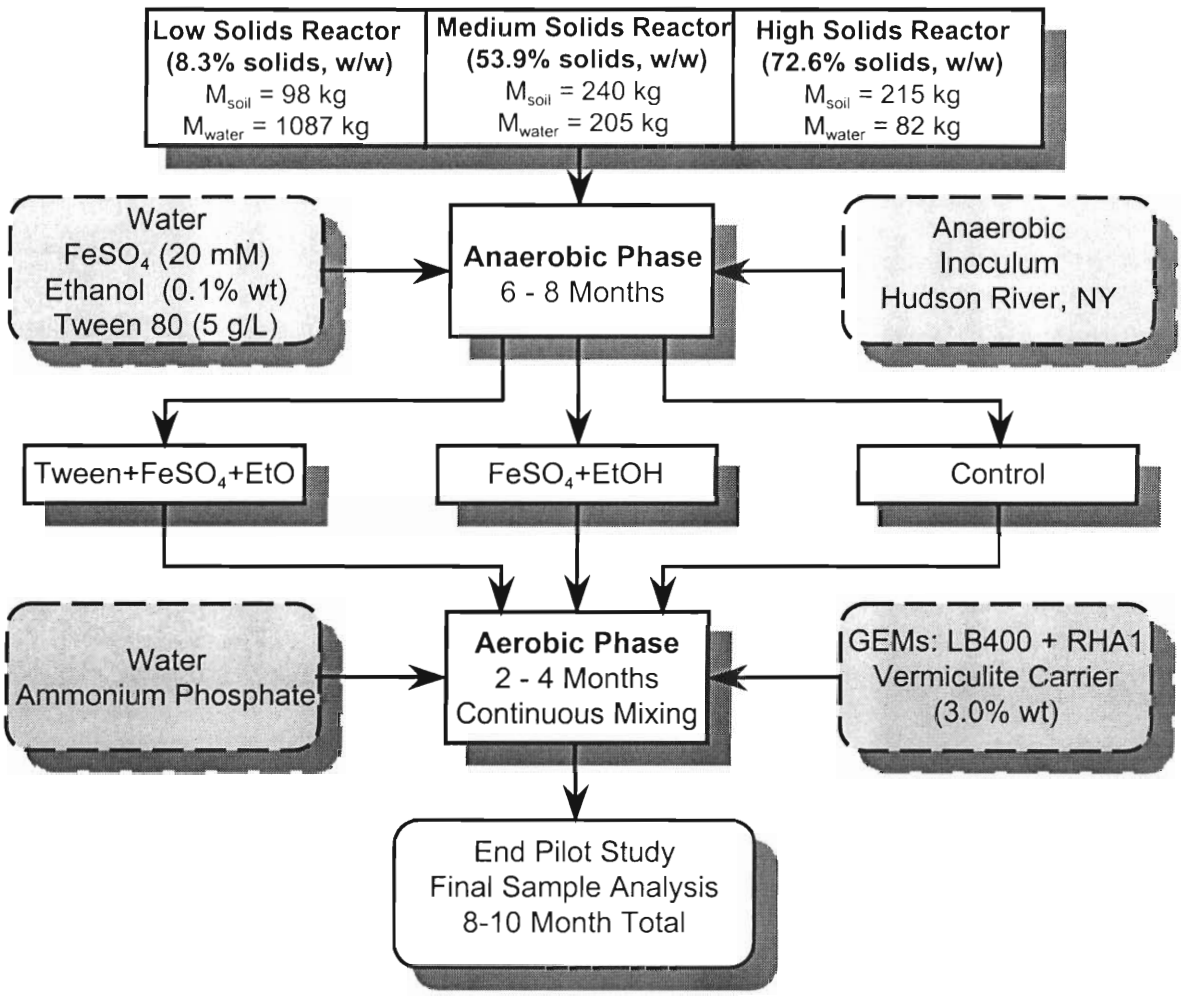

Figure 7: Flow diagram illustrating process streams for pilot-scale biotreatment of Rome soil.

Table 9. Results of Tween 80 and total organic carbon (TOC) analyses at time = 0 for the low- (LSR), medium- (MSR) and high- (HSR) solids reactors.

Sample ID	Location	Date	State	Analysis	Conc.	Units
LSR#1 (PCB Demo)	Grab Sample	5/22/01	L/S	Surfactant(Tween 80)	**3.05**	**mg/L**
LSR#1 (PCB Demo)	Grab Sample	5/22/01	L/S	TOC	**23.06**	**mg/g**
LSR#2 (PCB Demo)	Bottom Level	5/22/01	L/S	TOC	**5.40**	**mg/g**
LSR#3 (PCB Demo)	Bottom Level	5/22/01	L/S	TOC	**6.34**	**mg/g**
MSR#1(PCB Demo)	Grab Sample #1	5/22/01	S	Surfactant(Tween 80)	**1.04**	**mg/L**
MSR#1(PCB Demo)	Grab Sample #1	5/22/01	S	TOC	**10.39**	**mg/g**
MSR#1(PCB Demo)	Grab Sample #2	5/22/01	S	TOC	**9.73**	**mg/g**
MSR#1(PCB Demo)	Grab Sample #4	5/22/01	S/L	TOC	**10.51**	**mg/g**
MSR#2(PCB Demo)	Grab Sample #1	5/22/01	S/L	TOC	**6.82**	**mg/g**
MSR#2(PCB Demo)	Grab Sample #2	5/22/01	S	TOC	**15.42**	**mg/g**
MSR#3(PCB Demo)	Grab Sample #1	5/22/01	S/L	TOC	**0.80**	**mg/g**
MSR#3(PCB Demo)	Grab Sample #2	5/22/01	S	TOC	**8.35**	**mg/g**
HSR#1(PCB Demo)	Grab Sample #1	5/21/01	S	TOC	**7.36**	**mg/g**
HSR#1(PCB Demo)	Grab Sample #2	5/21/01	S	TOC	**12.77**	**mg/g**
HSR#1(PCB Demo)	Grab Sample #3	5/21/01	S	TOC	**9.90**	**mg/g**
HSR#2(PCB Demo)	Grab Sample #1	5/21/01	S	TOC	**5.35**	**mg/g**
HSR#2(PCB Demo)	Grab Sample #2	5/21/01	S	TOC	**5.47**	**mg/g**
HSR#2(PCB Demo)	Grab Sample #3	5/21/01	S	TOC	**3.50**	**mg/g**
HSR#3(PCB Demo)	Grab Sample #1	5/21/01	S	TOC	**7.52**	**mg/g**
HSR#3(PCB Demo)	Grab Sample #2	5/21/01	S	TOC	**5.03**	**mg/g**
HSR#3(PCB Demo)	Grab Sample #3	5/21/01	S	TOC	**5.27**	**mg/g**

Note:**TOC** was analyzed using *TOC-5050A with SSM-5000A* (SHIMADZU) in duplicate.
Soil samples for TOC analysis were dried in an oven at 55°C for 18 hours.
TOC values are based on per gram oven-dry soil.

Table 10. Results of specific surface area (SSA), total organic carbon (TOC) of the untreated (original) PCB-contaminated soil.

Sample ID	Location	Date	State	Analysis	Conc.	Units
PCB Soil	Rome, GA Site	5/22/01	S	Surfactant(Tween 80)	**ND**	**mg/L**
PCB Soil	Rome, GA Site	5/22/01	S	Metals	**below**	**mg/kg**
PCB Soil	Rome, GA Site	5/22/01	S	TOC	**6.27**	**mg/g**
PCB Soil	Rome, GA Site	5/22/01	S	N_2/BET Surface Area	**25.20**	**m^2/g**

Note: **Surface area** was analyzed using a Micromeretics *ASAP 2010* (Micromeritics) in duplicate. Soil samples for SSA analysis were dried in an oven at 105°C for 18 hours.

Table 11. Results ofmetals analysis of the untreated (original) PCB-contaminated soil.

Metal	C_{wet} (mg/kg)	C_{dry} (mg/kg)
B	18.27	21.27
As	19.17	26.99
Cd	4.27	6.17
Cr	62.87	90.25
Co	11.09	15.97
Cu	14.36	20.70
Pb	33.65	47.77
Mn	296.96	427.67
Mo	4.80	6.87
Ni	48.83	70.44
Se	321.93	457.47
Zn	199.15	287.37
K	231.80	281.65
Na	6.72	8.16
Mg	25.36	30.82
Ca	215.50	261.84
Fe	1.47	1.79
Al	870.32	1057.46

Note: As,B,Cd,Co,Cr,Cu,Hg,Mn,Mo,Ni,Pb,Se,Zn,Al,Fe,Mg,Ca, and K were analyzed using an *ICAP Tracer Analyzer* (Thermo Jarrell Ash Co).

Na was analyzed using an *AAnalyst 800* (PERKIN ELMER)

Digestion: As,B,Cd,Co,Cr,Cu,Hg,Mn,Mo,Ni,Pb,Se, and Zn were digested according to method 3050A in SW-846 (3rd ed.,1995).

Extraction: Exchangable metals (Al,Fe,Mg,Ca,K,Na) were extracted according to Pennell et al. (1991)

Table 12. Surfactant (Tween 80) and total organic carbon (TOC) analysis of grab samples colleced from low solids reactor (LSR), medium solids reactor (MSR), and high solids reactor (HSR) after 76 days of operation.

Sample ID	Sample	Analysis	Results
LSR#1	composite#1	Surfactant (Tween80)	**125.29mg/L**
LSR#1	composite#1	TOC	**27.16mg/g**
LSR#1	composite#2	Surfactant (Tween80)	**127.07mg/L**
LSR#1	composite#2	TOC	**29.65mg/g**
LSR#2	composite#1	TOC	**5.28mg/g**
LSR#2	composite#2	TOC	**5.92mg/g**
LSR#3	composite#1	TOC	**6.04mg/g**
LSR#3	composite#2	TOC	**5.75mg/g**
MSR#1	composite#1	Surfactant (Tween80)	**NA**
MSR#1	composite#1	TOC	**10.38mg/g**
MSR#1	composite#2	Surfactant (Tween80)	**NA**
MSR#1	composite#2	TOC	**10.80mg/g**
MSR#2	composite#1	TOC	**7.81mg/g**
MSR#2	composite#2	TOC	**8.11mg/g**
MSR#3	composite#1	TOC	**7.71mg/g**
MSR#3	composite#2	TOC	**7.51mg/g**
HSR#1	composite#1	Surfactant (Tween80)	**NA**
HSR#1	composite#1	TOC	**9.62mg/g**
HSR#1	composite#2	Surfactant (Tween80)	**NA**
HSR#1	composite#2	TOC	**9.33mg/g**
HSR#2	composite#1	TOC	**5.67mg/g**
HSR#2	composite#2	TOC	**8.43mg/g**
HSR#3	composite#1	TOC	**4.77mg/g**
HSR#3	composite#2	TOC	**6.31mg/g**

Note: No separate liquid phase in samples MSR#1 and HSR#1, and therefore, no Tween 80 analysis.

Table 13. Surfactant (Tween 80) and total organic carbon (TOC) analysis of grab samples colleced from low solids reactor (LSR), medium solids reactor (MSR), and high solids reactor (HSR) after 136 days of operation.

Sample ID	Sample	Analysis	Results
LSR#1	composite#1	Sufactant (Tween 80)	**66.86mg/L**
LSR#1	composite#1	TOC	**25.17mg/g**
LSR#1	composite#2	Sufactant (Tween 80)	**52.43mg/L**
LSR#1	composite#2	TOC	**24.74mg/g**
LSR#2	composite#1	TOC	**5.43mg/g**
LSR#2	composite#2	TOC	**5.97mg/g**
LSR#3	composite#1	TOC	**5.06mg/g**
LSR#3	composite#2	TOC	**5.65mg/g**
MSR#1	composite#1	Sufactant (Tween 80)	**NA**
MSR#1	composite#1	TOC	**9.42mg/g**
MSR#1	composite#2	Sufactant (Tween 80)	**NA**
MSR#1	composite#2	TOC	**9.82mg/g**
MSR#2	composite#1	TOC	**7.85mg/g**
MSR#2	composite#2	TOC	**7.62mg/g**
MSR#3	composite#1	TOC	**7.63mg/g**
MSR#3	composite#2	TOC	**7.61mg/g**
HSR#1	composite#1	Sufactant (Tween 80)	**NA**
HSR#1	composite#1	TOC	**7.68mg/g**
HSR#1	composite#2	Sufactant (Tween 80)	**NA**
HSR#1	composite#2	TOC	**9.77mg/g**
HSR#2	composite#1	TOC	**4.34mg/g**
HSR#2	composite#2	TOC	**5.45mg/g**
HSR#3	composite#1	TOC	**6.09mg/g**
HSR#3*	composite#2	TOC	**10.21mg/g**

Table 14. Surfactant (Tween 80) and total organic carbon (TOC) analysis of grab samples colleced from low solids reactor (LSR), medium solids reactor (MSR), and high solids reactor (HSR) after 197 days of operation.

Sample ID	Sample	Analysis	Results
LSR#1	composite#1		broken during shipping
LSR#1	composite#1	TOC	**26.22mg/g**
LSR#1	composite#2	Surfactant (Tween80)	**59.69mg/L**
LSR#1	composite#2	TOC	**31.19mg/g**
LSR#2	composite#1	TOC	**10.61mg/g**
LSR#2	composite#1	Tween 80	**ND**
LSR#2	composite#2	TOC	**8.92mg/g**
LSR#2	composite#2	Tween 80	**ND**
LSR#3	composite#1	TOC	**7.84mg/g**
LSR#3	composite#1	Tween 80	**ND**
LSR#3	composite#2	TOC	**7.79mg/g**
LSR#3	composite#2	Tween 80	**ND**
MSR#1	composite#1	Surfactant (Tween80)	**NA**
MSR#1	composite#1	TOC	**12.75mg/g**
MSR#1	composite#2	Surfactant (Tween80)	**NA**
MSR#1	composite#2	TOC	**12.48mg/g**
MSR#2	composite#1	TOC	**10.02mg/g**
MSR#2	composite#2	TOC	**9.47mg/g**
MSR#3	composite#1	TOC	**11.14mg/g**
MSR#3	composite#2	TOC	**10.05mg/g**
HSR#1	composite#1	Surfactant (Tween80)	**NA**
HSR#1	composite#1	TOC	**8.28mg/g**
HSR#1	composite#2	Surfactant (Tween80)	**NA**
HSR#1	composite#2	TOC	**10.44mg/g**
HSR#2	composite#1	TOC	**9.63mg/g**
HSR#2	composite#2	TOC	**7.82mg/g**
HSR#3	composite#1	TOC	**8.83mg/g**
HSR#3	composite#2	TOC	**8.36mg/g**

(VII) TECHNOLOGY TRANSFER

The SERDP PCB project will be moving to the pilot-scale testing phase during FY00. This process has resulted in some refocusing of this project to address field implementation and design issues, as well as economic analysis of PCB bioremediation technologies, and comparisons to conventional treatments such as incineration and landfill disposal. Two potential field sites were, Lake Ontario Ordinance Works (LOOW) and Picatinny Arsenal, with current activities focusing on PCB-contaminated soil from Picatinny Arsenal. Current bioreactor design efforts are being coordinated with Lance Hansen at WES and the WAM Corporation, a manufacturer of shaftless conveyer screws. These activities will lead to direct technology transfer opportunities with both the WAM Corporation and potential PCB bioremediation clients. Current technology transfer activities involve web page presentation of results, preparation of manuscripts for publication, and presentation of research findings at national meetings.

(VIII) PUBLICATIONS AND PRESENTATIONS

(1) Howell, D.P. 1999. Evaluation of Surfactants for the Enhancement of PCB Degradation. M.S. Thesis. Georgia Institute of Technology, Atlanta, GA.

(2) Howell, D.P. and K.D. Pennell. 1998. Evaluation of surfactants for the enhancement of PCB dechlorination. In Abstracts of the American Society of Microbial Ecology National Meeting, May 18-21, 1998, Atlanta, GA.

(3) Howell, D.P., Pennell, K.D. and Tiedje, J.M. 1999. Compatibility of PCB-degrading bacteria with selected nonionic surfactants. (in preparation).

(4) Pennell, K.D. and L.M. Abriola. 1997. Surfactant enhanced aquifer remediation: Fundamental processes and practical application. *In* Bioremediation: Principles and Practice, Volume 1. S.K. Sikdar and R.L. Irvine (eds.), Technomic Publ., Lancaster, PA pp. 693- 750.

(IX) STUDENTS SUPPORTED

Desiree P. Howell, M.S., 1999
Lingjun Kong, Ph.D. (expected 2004)
Charlotte Martin, M.S., 2001